RESEARCH ARTICLE

Patterns of habitat occupancy, genetic variation and predicted movement of a flightless bush cricket, *Pholidoptera griseoaptera*, in an agricultural mosaic landscape

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Abstract Habitat fragmentation has been generally regarded detrimental to the persistence of many species, especially those with limited dispersal abilities. Yet, when exactly habitat elements become functionally disconnected very much depends on the dispersal ability of a species in combination with the landscape's composition in which it occurs. Surprisingly, for many small and ground-walking generalists knowledge at what spatial scale and to what extent landscape structure affects dispersal is very scarce. Because it is flightless, the bush cricket *Pholidoptera griseoaptera* may be regarded susceptible to fragmentation. We applied habitat occupancy surveys, population genetic analyses and movement modelling to investigate the performance of *P. griseoaptera* in an agricultural mosaic landscape with suitable habitat patches of varying size and isolation. Despite its presumed dispersal limitation we could show that *P. griseoaptera* occupied the majority of suitable habitats, including small and isolated patches, showed a very low and non-significant genetic differentiation ($F_{ST} = 0.0072$) and, in the model, managed to colonize around 73% of all suitable habitat patches within one generation under weak and strong landscape-effect scenarios. We conclude that *P. griseoaptera* possesses the behavioural attributes (frequent

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inter-patch dispersal) necessary to persist in this landscape characterized by a patchy distribution of habitat elements. Yet, sound recommendations to landscape planning and conservation require more research to determine whether this represents a general behaviour of the species or a behavioural adaptation to this particular landscape.

Introduction

Agricultural landscapes in Europe have undergone major changes in land-use intensity and landscape structure resulting in an ongoing fragmentation of semi-natural habitats (Meeus 1993; Stoate et al. 2001). The fragmentation of habitats has been generally regarded detrimental to the persistence of many species, especially those with limited dispersal ability (Fahrig 2003; Hendrickx et al. 2009). Yet, whether fragmentation exerts negative effects on a particular species is closely related to its dispersal abilities (Hanski 1999). Whereas patch isolation or patch size may be of minor importance when species are highly vagile, species with more limited dispersal capacities are often strongly affected by these characteristics of landscape structure (Thomas 2000). At what extent reduced size and increased isolation of habitats resulting from fragmentation start to negatively affect species with limited dispersal abilities, however, often remains unknown.

Reduced size and increased isolation of habitat elements have generally been found to lead to smaller population sizes and decreased inter-patch dispersal (Hanski 1999). Contrary to stochastic extinctions due to small population sizes, other negative effects of changes in landscape structure will not be immediately detectable but may only occur gradually and after a certain time-lag. For instance, decreased inter-patch dispersal may lead to increased levels of inbreeding, smaller effective population sizes, and loss of genetic variation. Reduced fitness (Jimenez et al. 1994; Saccheri et al. 1998; Reed and Frankham 2003) and in the long term increased local extinctions

in spatially structured populations (Saccheri et al. 1998; Nieminen et al. 2001) will be the potential consequences.

Many species inhabiting agricultural mosaic landscapes with a scattered distribution of suitable habitats are regarded generalists that are capable of flexibly adjusting their requirements and behaviour to changes in their environment. Surprisingly, however, for many small and ground-walking generalists, such as spiders, ground beetles or various bush crickets, knowledge on how landscape structure affects dispersal, genetic variability and population persistence is very scarce (Hein et al. 2003; Sander et al. 2006; Diekötter et al. 2007; Matern et al. 2008). In addition, the existing studies on the genetic structure of flightless ground beetles vary in their spatial extents and the scales at which significant population differentiation was found (Niehues et al. 1996; Brouat et al. 2003; Keller et al. 2004; Sander et al. 2006). Therefore, it remains largely unknown whether current distributions of these small and ground-walking species, in particular flightless bush crickets, actually reflect dispersal abilities that allow for their long-term persistence in landscapes with a scattered distribution of suitable habitat or only mirror dispersal regimes from the past.

Here, we followed complementary approaches at local and regional spatial scales in order to investigate the capability of a flightless insect species to persist in an agricultural landscape showing a gradient from large and connected to small and isolated suitable habitat elements. Distributional data on *P. griseoaptera* as well as data on the genetic population structure of the species were used in combination with an individual-based, spatially-explicit movement model to find out whether in an agricultural landscape with a scattered distribution of the species' preferred habitat this flightless bush cricket (a) occupies only large and well connected or also small and isolated habitat patches, (b) shows restricted or frequent gene flow among spatially structured habitat elements at the local scale, and (c) is able to colonize the majority of suitable habitat patches at a regional scale.

Long distance dispersal, gene flow levels over time (see Crochet 1996) and local extinctions as well as the extent to which they are affected by landscape structure are particularly difficult to detect in classical movement studies. Therefore, landscape genetics and

movement modelling based on empirical data repre-sent very valuable methods to extend our knowledge on the nature of dispersal, gene flow regimes and population persistence in spatially structured popula-tions (Waser and Strobeck 1998; Vos et al. 2001b; Balloux and Lugon-Moulin 2002; Arens et al. 2007; Storfer et al. 2007). We argue that an understanding of the processes underlying the observed patterns of dispersal and genetic structure of populations in fragmented landscapes is important to develop eco-logically scaled landscape indices (Vos et al. 2001b) that in turn may contribute to more sustainable landscape planning (Opdam et al. 2002).

Materials and methods

Study species

The dark bush cricket *Pholidoptera griseoaptera* (De Geer, 1773) (Orthoptera: Tettigoniidae) is mainly distributed in Central and Eastern Europe and extends to the northern parts of Britain and Scandinavia as well as the Mediterranean (Maas et al. 2002). The species is strongly associated with woody structures with a grass, tall herb or shrub layer and is commonly found in forest clearings as well as along woodland edges and in hedgerows (Samietz 1995). *P. gris-eoaptera* can sometimes also be found in various other habitat types in agricultural landscapes such as extensively used semi-natural grasslands and reed beds (Samietz 1995) if these are near woody vegetation.

   P. griseoaptera is polyphagous, feeding on plants (*Rubus* sp., *Taraxacum officinale*, *Urtica dioica*) as well as on insects such as caterpillars, flies and spiders. It has a biennial life cycle. The eggs are laid during summer and autumn and hatch in spring of the second year. The nymphs go through seven instars before they reach the adult stage. Imagos measure 13–18 mm in length, occur from mid July until Novem-ber, and are active during both day and night. The species is unable to fly and fully winged (macropter-ous) individuals have never been observed.

Landscape characteristics

The study took place in the area of the Nussbaumer Seen (47°35′N, 8°48′E), a plateau situated in the southwestern part of the Bodensee area in the Canton of Thurgau (∼500 m a.s.l.; Fig. 1a) in Switzerland. The area is characterized by a mean annual temper-ature of 10°C and an average annual precipitation of 700–1,100 mm. A large proportion of the area is under agricultural cultivation, but there are many patches of woodland embedded in the agricultural matrix. These woody elements, representing suitable habitat for *P. griseoaptera*, cover 25.1% of the area. Woody elements in the area of the Nussbaumer Seen show an edge density of 116 m/ha and are on average 23.3 m apart from each other. Intensification of agricultural practices during the last century has led to a decrease of semi-natural landscape elements—including the woody structures—of ∼35% between 1940 and 1960 (European Union (EU) research project "Greenveins", unpublished data). Today's intensity of agriculture in the area, measured as nitrogen input on arable crops and on permanent grassland, livestock density, and pesticide applica-tions, has been estimate to be of a medium level in the European perspective (cf. Herzog et al. 2006, landscape test site (LTS) H-NUB).

   Land cover was digitised from recent true colour orthophotos, which had a spatial resolution of less than 1 m, in combination with topographical maps using ArcGIS 8.1 (ESRI). Landscape elements were defined using a scheme based on the European EUNIS habitat classification system (Davies and Moss 1999) and delineated in accordance to a specific landscape mapping protocol (Bailey et al. 2007). Digitised elements were either discrete patches (e.g. arable fields, meadows and woodlands) or linear features (e.g. grassy margins and littoral zones alongside water bodies, field margins, road verges, hedgerows and tree rows). Ground truthing was undertaken to ensure map reliability. For the use in the pre-existing simulation model for individual animal movement SMALLSTEPS (cf. Snep et al. 2006) each patch or linear feature was assigned to a particular group of land-use types characterized by a set of group-specific movement parameters (Table 1, Appendix 1—Electronic Supple-mentary Material). Adjacent habitat elements belong-ing to the same habitat group were merged to clusters.

Presence–absence surveys

In 2002 we surveyed presence-absence patterns of *P. griseoaptera* along all woody habitat structures at
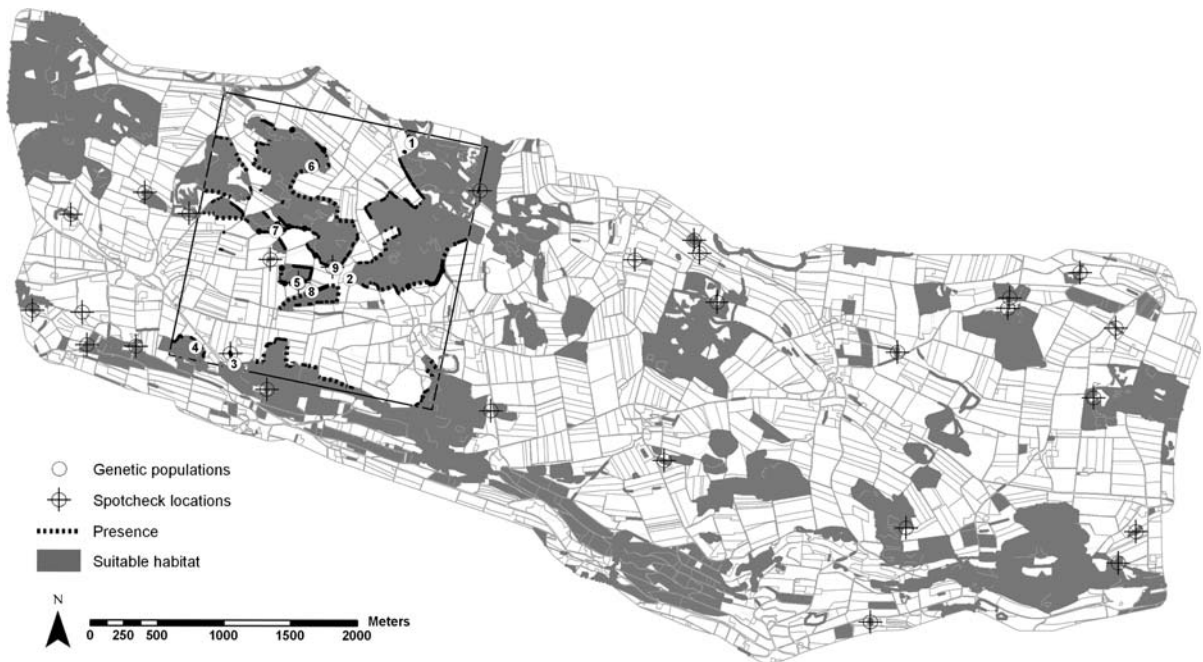
Fig. 1 Distribution of suitable habitat for *Pholidoptera griseoaptera* (*dark grey*), presences of *P. griseopatera* in a 2 × 2 km subarea in 2002 (*black dots* and *lines*), spotcheck locations in 2003 (*target symbols*), and populations of genetic sampling in 2003 (*numbered circles*) in the study area "Nussbaumer Seen", Canton of Thurgau, Switzerland

a local scale within the centre part of our study area (Fig. 1). In this area, 4 km² in size, *P. griseoaptera* was acoustically surveyed during evening hours in August 2002 by walking along the edges of all woody habitat elements. Woody habitat elements that were classified unoccupied by *P. griseoaptera* during a first survey were visited a second time.

In 2003 we investigated presence–absence of *P. griseoaptera* in thirty woodland elements at a regional scale across the whole study area of 16 km² (Fig. 1). All habitat elements selected were generally regarded suitable for *P. griseoaptera* with regard to the available resources and vegetation structure. The elements differed, however, in size and isolation. Ten habitat elements each were (a) small and isolated, (b) medium sized with elements of the same habitat type in their surrounding or (c) rather large and well connected. The selected habitat elements were visited and both acoustically as well as visually inspected for the presence of *P. griseoaptera* during evening hours with warm and dry weather conditions in early August 2003. Locations with *P. griseoaptera* not being recorded at the first occasion were revisited a second evening.

Population genetics

Genetic samples were collected from nine locations in September and October 2002 within an area of 2 × 2 km. The sampling locations were selected on the basis of the surveyed distribution of *P. griseoaptera* and of local landscape characteristics. Sampling locations were selected along a gradient of increasing inter-patch distances with or without shares of unsuitable matrix between pairs of locations (Fig. 1). The preferred sample size of 30 individuals per sampling location could not always be achieved due to small population sizes in various locations. In total, 241 hind legs of *P. griseoaptera* were collected (one per animal), originating from 112 males and 129 females. Individual legs were immediately put in 70% ethanol and stored until processing.

Hind leg samples were first washed with deionised water to rinse away any ethanol. DNA was isolated either with a standard animal tissue DNA extraction protocol using proteinase K and phenol chloroform extraction or with the InstaGene Matrix (Biorad) with addition of 10 μl 20 mg/ml proteinase K (Cooper and Hewitt 1993). After DNA extraction samples were

Table 1 Transition probabilities between patchy (polygon) and linear land-use types used to simulate strong, moderate and no landscape effects in SmallSteps

	From\to	Polygon barrier	Polygon road	Polygon path	Polygon grass	Polygon arable	Polygon move	Polygon forest	Polygon hedge	Polygon forest
Strong	Polygon barrier	1	1	1	1	1	1	1	1	1
	Polygon road	1	1	1	1	1	1	1	1	1
	Polygon path	1	1	1	1	1	1	1	1	1
	Polygon grass	0.25	0.25	0.25	1	1	1	1	1	1
	Polygon arable	0.25	0.25	0.25	1	1	1	1	1	1
	Linear move	0.05	0.05	0.05	0.25	0.25	1	1	1	1
	Linear forest	0.05	0.05	0.05	0.25	0.25	1	1	1	1
	Linear hedge	0.05	0.05	0.05	0.25	0.25	1	1	1	1
	Polygon forest	0.05	0.05	0.05	0.25	0.25	1	1	1	1
Moderate	Polygon barrier	1	1	1	1	1	1	1	1	1
	Polygon road	1	1	1	1	1	1	1	1	1
	Polygon path	1	1	1	1	1	1	1	1	1
	Polygon grass	0.5	0.5	0.5	1	1	1	1	1	1
	Polygon arable	0.5	0.5	0.5	1	1	1	1	1	1
	Linear move	0.1	0.1	0.1	0.5	0.5	1	1	1	1
	Linear forest	0.1	0.1	0.1	0.5	0.5	1	1	1	1
	Linear hedge	0.1	0.1	0.1	0.5	0.5	1	1	1	1
	Polygon forest	0.1	0.1	0.1	0.5	0.5	1	1	1	1
No	Polygon barrier	1	1	1	1	1	1	1	1	1
	Polygon road	1	1	1	1	1	1	1	1	1
	Polygon path	1	1	1	1	1	1	1	1	1
	Polygon grass	1	1	1	1	1	1	1	1	1
	Polygon arable	1	1	1	1	1	1	1	1	1
	Linear move	1	1	1	1	1	1	1	1	1
	Linear forest	1	1	1	1	1	1	1	1	1
	Linear hedge	1	1	1	1	1	1	1	1	1
	Polygon forest	1	1	1	1	1	1	1	1	1
Average step length [m]		65.000	65.000	10.000	6.911	45.750	5.396	7.153	5.396	7.153
SD turning angle [rad]		0.010	0.010	0.100	4.544	0.393	4.223	4.184	4.223	4.184

The same average step length and standard deviation of turning angle for each land-use type applied for all three landscape scenarios. Polygon Barrier and Linear Move are composite land-use types that in the case of the latter encompassed grassy margins along fields, tracks, roads and ditches (see Appendix 1—Electronic Supplementary Material for more detail)

analysed using seven previously described microsatellite loci (WPG2-15, 2–16, 2–39, 10–1, 9–1, 7–11, 1–28; Arens et al. 2005). Amplification and detection of SSR loci were described previously (Arens et al. 2005).

Unless noted otherwise, analyses were conducted using TFPGA 1.3 (Miller 1997). Departures from Hardy–Weinberg equilibrium (HWE) were tested for each of the seven loci using an exact test following the Markov Chain method (Guo and Thompson 1992). Associations between genotypes at pairs of loci in each sample were tested with help of a G-test in FSTAT Version 2.9.3 (Goudet 2001). To assess relative amounts of genetic variation in each population, mean number of alleles (A), allelic richness (R), unbiased expected heterozygosity (H_E), and observed heterozygosity (H_O) were calculated. Allelic richness and genetic diversity were calculated as implemented in FSTAT.

Wright's *F*-statistics were calculated using the method of Weir and Cockerham (1984) and used to quantify the magnitude of differentiation in allele frequencies among populations either in pairwise or overall comparisons. Pairwise F_{ST} estimates between sampled populations were obtained from Genepop on the Web 3.4 (Raymond and Rousset 1995b) and tested for significance using an exact test in TFPGA. Because of multiple pairwise comparisons the critical probability for each test was adjusted using the sequential Bonferroni procedure (Rice 1989). Genetic differentiation between populations was tested for significance using a contingency table approach (Fisher's R×C test). The probability of an observed allelic distribution was approximated using a Markov Chain Monte Carlo approach (Raymond and Rousset 1995a). Evidence of a recent population bottleneck was assessed for each population with BOTTLE-NECK (Cornuet and Luikart 1996) using the Wilcoxon signed-rank test and the qualitative descriptor of the allele frequency distribution (mode-shift indicator).

Isolation by distance was examined by applying a partial Mantel test with 1,000 permutations in the Vegan package of R (Version 2.7.2). Distances between *P. griseoaptera* populations were calculated either as the geographical distance between centres of sampling 'as the crow flies' (as used by e.g. Arens et al. 2007), the total sum of costs along the least-cost paths or the lengths of the least-cost paths between each pair of sampling locations. Least-cost distances were calculated using the ArcView Extension PATHMATRIX (Ray 2005). For doing so, the vector map was transformed into a grid map with a grain size of 1×1 m. We assigned friction values of 1 to suitable habitat types and 2 to all other habitat types (Appendix 1—Electronic Supplementary Material). All three, the geographical distance, the total sum of costs along the least-cost paths and the lengths of the least-cost paths between each pair of sampling locations, were used in the isolation by distance analysis. Distances were measured in a GIS-environment (ArcView 3.3, ESRI, 2000).

Movement model

Using movement data for *P. griseoaptera* obtained in field experiments in the same as the above landscape in Switzerland (Diekötter et al. 2005; 2007), we applied the vector-based movement model SMALL-STEPS (http://purl.oclc.org/net/Alterra/movement and Snep et al. 2006) to simulate the movement of individual animals in the studied landscape. Within polygons of a given vegetation type, movement is represented by a correlated random walk (CRW, see Kareiva and Shigesada 1983) defined by distributions of step length (or, equivalently, velocity) and turning angle. In linear landscape elements, represented as arcs, persistent random walk (PRW) movement was simulated in a comparable way, using a step length distribution and a turning probability instead of a turning angle distribution. Turning probability was derived from turning angle distribution in comparable polygon-type habitat as one minus the total probability of turning less than 90°.

Experimental data (e.g. capture-mark-resight data) obtained at fixed intervals in the field can be directly used to estimate probability distributions of velocity and turning angles (Turchin 1998). Based on observed 24-h displacements of a total of two hundred released animals that were recorded over a period of 10 consecutive days in the field (Diekötter et al. 2005; 2007), we calculated an appropriate step length using the model probability distribution: $P_{length}(l) = \frac{1}{\lambda} \exp\left(-\frac{l}{\lambda}\right)$, with λ being the expected step length. As turning-angle distribution we employed a zero-mean Gaussian distribution with standard deviation σ. For small σ this distributional form describes a (strongly) correlated movement in which the change of direction between consecutive steps is small. For large enough σ this distribution describes completely random motion.

Since the applied model landscapes consisted of more than one habitat type, the model needed to specify the behavior at habitat boundaries. We defined transition probabilities as the probabilities of crossing boundaries between each possible pair of habitat types. In case of 'failure to cross' we assumed simple, billiard-ball like reflection. For this study, we employed three different sets of transition probabilities simulating no, moderate and strong landscape effects. Absence of landscape effects was simulated by very high transition probabilities between all types (no barriers), strong effects by very low transition probabilities (strong barriers) and moderate effects by intermediate transition probabilities (Table 1).

In each simulation, the starting and arrival points were in woody habitat elements. In total there were

348 habitat clusters defined in the studied landscape. From each habitat cluster 10,000 individuals were released at random locations. As we observed juveniles to contribute substantially to movement and dispersal (Diekötter et al. 2005), released individuals were allowed to reallocate themselves according to the specified movement parameters for 70 time steps. With one time step representing 1 day, this amounts to an estimated lifespan of *P. griseoaptera* (4 later instars of 7 days each + 6 weeks of average adult life span).

The SMALLSTEPS model estimated patch encounter rates a_{ij}, also referred to as patch accessibility (Heinz et al. 2005): the probability for a disperser leaving (natal) patch i to encounter patch j. The a_{ij} values together constitute an alternative measure of patch connectivity, taking into account the landscape in between the patches of reproduction habitat.

To assess emigration rates and to translate arrival accessibility into realistic numbers of immigrants into each patch, we estimated population densities per patch by multiplying the patch's perimeter by 0.5 ind/m² (Detzel 1985). The number of emigrants from each patch i thus amounts to $E_i = 0.5 \cdot \text{PERI}_i$. Thus, the number of emigrants successfully dispersing from patchi i to patch j amounts to $E_{ij} = E_i \cdot a_{ij}$. Accordingly, the number of immigrants into a patch i, originating from patch j amounts to $I_{ij} = E_j \cdot a_{ji}$. Because of the small sample size ($n = 9$) and not normally distributed variables we used Kendall's tau as a rank-based measure of association between population genetic parameters such as allelic richness (R), observed heterozygosity (H_O), gene diversity (H_{sk}), and inbreeding coefficient (F_{IS}) and E_i as a proxy of the population size of the target patch i, $\dfrac{\sum_{j,j\neq i}^{n} E_j \cdot a_{ji}}{E_i}$, for the scenario with weak and strong landscape effects (too few populations received immigrants under the no-effect scenario). We did not relate pairwise F_{ST} values and flows of individuals between populations because too few individuals directly exchanged between the focal populations within one generation.

Because gene flow may not only occur via direct inter-patch dispersal but also via indirect pathways including several spatially separated populations across several generations, we also investigated whether the nine populations selected for population genetic analyses belonged to the same ecological network. Networks were created based on the value of incoming and outgoing flows I_{ij} (Vos et al. 2008). If the sum of incoming flows into patch i from all patches in a network exceeds a threshold value T, patch i belongs to the same network. Likewise, if the sum of all flows going out from patch i to all patches in a network exceeds T, patch i also belongs to this network. T is defined as the total number of immigrants (per dispersal period) that is required for a patch to be functionally connected to a network. Here, we set T to one according to the one-migrant-per-generation-rule in conservation and management (Mills and Allendorf 1996).

Results

Presence–absence surveys

Area-wide presence-absence surveys in the central part of the study area in 2002 found *P. griseoaptera* to be present along the edges of 16 (76%) of the total 21 suitable habitat elements or clusters in this area (Fig. 1). In 2003, presence-absence survey at each of ten habitat elements of poor, medium and high quality in terms of habitat size and isolation revealed a similar pattern. *P. griseoaptera* occupied all thirty locations visited in the study area, independent of their size or isolation (Fig. 1).

Population genetics

Genetic variation was estimated by genotyping a total of 241 individuals from nine populations ($n = 16–30$) at seven microsatellite loci. All seven microsatellite loci were polymorphic in all populations (Table 2). The total number of alleles across all populations ranged from 5 to 37 per locus with the highest genetic variation at locus WPG1-28 (Table 2). This locus and locus 2–39 showed a departure from Hardy–Weinberg equilibrium in all but two populations (Table 2) although the departure of the latter marker was not found in another population study (FH, unpublished data). The departures could be due to the presence of null alleles, but we did not find evidence for that in the form of a higher percentage of individuals without amplification. Since exclusion of loci from the analysis did not

Table 2 Diversity of allelic variation across loci. n = number of samples per sample site

Population	n	Number of alleles							Heterozygosity (expected/observed)						
		1–28	2–15	2–16	2–39	7–11	9–11	10–1	1–28	2–15	2–16	2–39	7–11	9–11	10–1
1	30	14	9	5	3	7	3	3	0.84/0.42***	0.77/0.67	0.62/0.54	0.53/0.09***	0.76/0.62	0.57/0.50	0.29/0.33
2	16	14	6	4	3	7	3	2	0.90/0.56**	0.75/0.73	0.58/0.38	0.55/0.18**	0.75/0.69	0.60/0.69	0.27/0.31
3	30	11	7	6	2	8	4	2	0.76/0.47**	0.78/0.70	0.62/0.63	0.48/0.25	0.71/0.67	0.56/0.50	0.28/0.27
4	16	13	6	4	2	7	4	2	0.90/0.44***	0.83/0.80	0.42/0.38	0.50/0.11*	0.80/0.75	0.62/0.69	0.23/0.25
5	30	17	7	7	2	8	3	3	0.88/0.50***	0.66/0.73	0.56/0.54	0.39/0.06***	0.77/0.79	0.49/0.53	0.32/0.17**
6	30	18	6	5	5	9	4	2	0.84/0.53***	0.68/0.70	0.55/0.48	0.42/0.35*	0.78/0.70	0.58/0.70	0.26/0.23
7	29	16	7	6	4	9	4	3	0.86/0.71***	0.64/0.75	0.62/0.68	0.58/0.41*	0.73/0.72	0.45/0.38	0.22/0.24
8	30	11	5	6	2	10	5	2	0.82/0.40	0.64/0.68	0.50/0.48	0.32/0.04***	0.76/0.70	0.53/0.50	0.13/0.13
9	30	15	7	5	4	9	3	2	0.8/0.57**	0.6/0.65	0.7/0.65	0.4/0.11***	0.8/0.70	0.5/0.36	0.1/0.14
Total	241	37	6	8	6	12	6	5							
Mean		14.3	6.7	5.3	3.0	8.2	3.7	2.3	0.85/0.51***	0.71/0.70	0.58/0.54	0.46/0.19***	0.75/0.70	0.54/0.52	0.23/0.23

Results of sample by locus and overall locus, and sample tests of conformance to Hardy–Weinberg expectations are provided

Significant deviations from Hardy–Weinberg at P-levels 0.05 (*), 0.01 (**), 0.001 (***)

alter the general results, we retained them for the further genetic analysis. Allelic richness (R) i.e. number of alleles per locus standardized to smallest number of individuals per site was 3.04–3.43 (Table 3). The observed heterozygosity (H_O) per locus ranged from 0.42 to 0.56 (average H_O: 0.49; Table 3). The inbreeding coefficient F_{IS} was significant ($F_{IS} = 0.173$, 95% CI 0.029–0.337) indicating significant inbreeding levels. None of the populations showed any signs of possible recent bottlenecks.

Investigating population differentiation revealed that the distribution of alleles over loci was not identical across sample sites ($\chi^2 = 61.04$, $P < 0.001$). Three of seven loci showed similar distributions of alleles among populations. F_{ST} analysis across all populations and loci showed a very low and non-significant differentiation ($\theta = 0.0072$, 95% CI -0.0009 to 0.0150). Pairwise F_{ST} values ranged from 0.000 to 0.040 (Table 4). Only 5 out of 36 pairwise comparisons were significant (Table 4).

Isolation by distance was analysed using geographical distance (i.e. measured as the crow flies) and ecologically meaningful distances between populations. Based upon the geographical distances no effect of isolation by distance was apparent ($r = 0.197$, $P = 0.165$). The length of the least cost path between populations, which was on average 20% longer than geographical distance, did not have a significant effect on genetic distances either ($r = 0.1856$, $P = 0.163$). The correlation of genetic distance with the total sum of costs along the least-

Table 3 Genetic variation within populations based on seven microsatellite markers

Population	H_E H_O	R	H_{sk}	F_{IS}
1	0.63/0.45	3.30	0.63	0.277
2	0.63/0.50	3.43	0.63	0.204
3	0.60/0.50	3.14	0.60	0.171
4	0.61/0.49	3.38	0.62	0.212
5	0.58/0.47	3.31	0.58	0.188
6	0.59/0.53	3.25	0.59	0.101
7	0.58/0.56	3.36	0.58	0.042
8	0.53/0.42	3.04	0.53	0.209
9	0.56/0.46	3.26	0.57	0.184
Mean	0.59/0.49	3.28	0.59	0.173

H_E expected heterozygosity, H_O mean observed heterozygosity, R allelic richness, H_{sk} gene diversity and F_{IS} per population

Table 4 Pairwise F_{ST} values between populations

Population	1	2	3	4	5	6	7	8	9
1	0								
2	−0.009	0							
3	−0.007	−0.003	0						
4	0.001	−0.011	0.002	0					
5	0.007	−0.003	−0.001	0.013	0				
6	**0.018**	−0.003	**0.011**	0.014	0.003	0			
7	0.018	0.015	0.010	0.040	−0.004	**0.016**	0		
8	**0.019**	0.001	0.004	0.021	−0.010	−0.002	0.004	0	
9	**0.020**	0.013	0.012	0.036	−0.008	0.015	0.001	0.000	0
Overall	0.0072								

Values given in bold are significant (exact test) after sequential Bonferroni correction

cost paths was marginally significant ($r = 0.293$, $P = 0.057$). However, this relationship strongly depended on the outermost population in the south-western corner of the sample area, which was separated from the inner cluster of sample sites by a paved road and comparatively large areas of agricultural land, and diminished when leaving out this population ($r = 0.237$, $P = 0.163$).

Movement model

The predicted landscape-wide rate of successful inter-patch dispersal of *P. griseoaptera* differed among the three landscape scenarios. Of all individuals virtually released within suitable habitat, 37.8, 13.0 and 10.3% successfully dispersed to suitable habitat elements under the scenarios of no, moderate and strong landscape effects, respectively. Although the scenario of no landscape effects showed the highest number of dispersing individuals these individuals arrived in 26.2% of all suitable habitat patches only, whereas under the weak- and strong-landscape-effect scenarios dispersers reached 72.7 or 73.6% of all suitable patches, respectively. On average, these colonized suitable habitat patches under the no-landscape-effects scenario were connected by dispersal to another 7.2 suitable habitat patches. Under the weak or strong scenario colonized suitable habitat patches were linked by dispersal with an average of 5.7 or 4.8 patches, respectively.

Consistent with the non-significant genetic differentiation among populations, for all scenarios—no, weak and strong landscape effects—the network analysis revealed a contiguous network of all nine genetically analysed populations when setting the connectivity threshold T to one exchanging individual per population. Direct exchange of individuals, however, was predicted among the central and proximate populations two, five, eight and nine, only (see Fig. 1).

There were no significant associations between E_i and the estimated population genetic parameters ($P \geq 0.075$).

Discussion

Despite the species being flightless and the scattered distribution of its main habitat, our results suggest that the bush cricket *P. griseoaptera* is fully capable of persisting in the studied agricultural mosaic landscape. Presence–absence surveys, genetic population analyses and movement modelling all indicated that *P. griseoaptera* exhibits successful inter-patch dispersal in this landscape and thereby succeeds in maintaining viable populations in almost all suitable habitat patches of varying size and isolation.

Generally, populations inhabiting small and isolated habitat patches are assumed to face a higher risk of extinction (Hanski 1999). Also, isolated habitat patches have a smaller chance of getting recolonized than more connected ones (MacArthur and Wilson 1967). Therefore, in a presence/absence survey one would expect small and isolated habitat patches to be unoccupied more often than large and connected ones. Yet, in our study, acoustically surveying the

presence of *P. griseoaptera* in an area of 4 km^2 revealed a virtually complete occupancy of suitable habitat elements. Not only was *P. griseoaptera* present along most suitable margins of large and well connected forest elements but also at edges of smaller and more isolated forest patches as well as hedgerow fragments. Acoustic spot checks for the presence of *P. griseoaptera* confirmed this occupancy pattern at a regional scale across an area of 16 km^2.

The fraction of occupied habitat patches has been shown to be a useful predictor of metapopulation viability (Hanski and Thomas 1994; Vos et al. 2001b) and an occupation threshold of 0.5 has been suggested to ensure sufficient recolonisations for long-term metapopulation persistence (Vos et al. 2001b). The very high habitat occupancy observed in this study contrasts with the expectation of unoccupied habitat patches as predicted by metapopulation theory (Hanski 1999). Yet, it suggests that *P. griseoaptera* possesses high dispersal abilities and across most habitat elements forms a continuous population rather than a metapopulation in this studied agricultural mosaic landscape.

Metapopulations are not only expected to show occupied and unoccupied habitat patches but also high levels of genetic differentiation among subpopulations due to reoccurring extinction events and restricted gene flow among subpopulations (Harrison and Hastings 1996; Uimaniemi et al. 2000; Williams et al. 2003). In contrast, a lower genetic differentiation among existing subunits would be expected in spatially structured populations due to more frequent inter-patch dispersal. Thus, a non-significant and very low population differentiation ($F_{ST} = 0.0072$) as observed for *P. griseoaptera* in the study area indicates frequent exchange of individuals among different habitat elements in a spatially structured population.

Because of time-lags in species' responses to landscape change (e.g. Holzhauer et al. 2006; Metzger et al. 2009), however, non-significant population differentiation and missing correlations between genetic and landscape structure may not necessarily result from frequent gene flow but just too little time for new equilibria to appear. By using a model-based clustering method (STRUCTURE, results not shown), we were able to rule out time-lags in the bush cricket's response to habitat fragmentation as a structure of four to five clusters was shown to be most

plausible. Individuals of the same population often showed inferred ancestry from different clusters. The significant correlation between the total costs of dispersal and population differentiation represented additional evidence for a synchronous ecological response of *P. griseoaptera* to landscape structure.

High costs of dispersal also seem to be reflected in the few significant pairwise population differences observed in this study. These involved the two outermost populations (1 and 3), in the north-eastern and south-western corners of the sample area, which are both separated from the inner cluster of sample sites by a paved road and by comparatively large areas of agricultural land. A negative effect of roads on inter-patch dispersal of animals has been previously shown (Gerlach and Musolf 2000; Vos et al. 2001a; Keller and Largiadèr 2003), whereas an apparently negative effect of increasing shares of agricultural land on inter-patch dispersal may result from unsuitable microclimatic conditions (Krooss and Schaefer 1998), insufficient food resources (Krooss and Schaefer 1998), an increased risk of predation (Lang et al. 1999), or direct deleterious effects in the agricultural matrix (Haughton et al. 1999; Kromp 1999). Regarding that no isolation-by-distance was apparent when using (minimum) geographical distances or the lengths of the least-cost paths between each pair of sampled populations, our results suggest that dispersal between populations may not be affected by distance per se but that increased costs for overcoming unsuitable habitat and physical barriers reduce effective dispersal (cf. Vos et al. 2001a).

Using the individual-based and spatially explicit movement model SMALLSTEPS in evaluating the permeability of our study area for dispersing individuals of *P. griseoaptera*, we could show that—depending on the strength of boundary effects modelled under the three different scenarios—up to 73.6% of all suitable habitat patches had been colonized within only one generation. This concurs with the observed area-wide occurrence of *P. griseoaptera* and supports the notion based on the genetics that the studied landscape appears rather permeable for this flightless bush cricket. This permeability seems to result from a combination of landscape characteristics and behavioural traits of *P. griseoaptera*. Comparing the high percentage of colonized habitat patches under the landscape

scenario with strong boundary effects with a value as low as 26.2% under the scenario with low boundary effects highlights the directing effect of habitat boundaries on moving individuals and its associated impact on landscape connectivity (Tischendorf and Wissel 1997; Berggren et al. 2002; Snep et al. 2006). This directing effect of habitat boundaries is supported by the high shares of suitable woody habitat in the studied landscape. However, our model results also suggest that in a landscape characterised by a high number of spatially separated suitable habitat elements like the studied one, a moderate to low readiness to leave suitable habitat in combination with a fast and straight movement once entered unsuitable habitat are the decisive characteristics that enable *P. griseoaptera* to persist in the studied landscape.

Together, the consistent presence of *P. griseoaptera* in habitat elements of different size and isolation as observed by spot checking, the very low and non-significant differentiation revealed in the population genetic analysis and the high colonization rates predicted by movement modelling suggest that *P. griseoaptera* possess higher dispersal abilities than might have been expected for this small and flightless generalist of the agricultural landscape. However, given the generally observed heritability of mobility traits (Roff and Fairbairn 2001) and the selective force of landscape configuration (Gandon and Michalakis 1999; Hill et al. 1999; Hanski et al. 2004), it must be kept in mind that movement characteristics may be landscape-dependent and that rapid fragmentation in formerly well-connected landscapes or habitat loss below a certain threshold may pose a serious threat to ground-walking species such as *P. griseoapatera*.

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